Drug Design, Development and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Development of HPLC-MS/MS Method for Simultaneous Detection of Esketamine and Norketamine: Application to Pharmacokinetics Drug Interactions Affected by Dexmedetomidine

Wei Zhou[1], Zhe Guo[1], Xianghan Zhang[2], Wenjiong Wang[2], Zhongfeng Zheng[3], Xaingjun Qiu[2,3]

[1]Department of Anesthesiology, Nanyang City Central Hospital, Nanyang, Henan, 473009, People's Republic of China; [2]College of Basic Medicine and Forensic Medicine, Henan University of Science and Technology, Luoyang, Henan, 471023, People's Republic of China; [3]Department of Forensic Toxicology, Henan Yicheng Judicial Appraisal Center, Zhengzhou, Henan, 450052, People's Republic of China

Correspondence: Xaingjun Qiu, College of Basic Medicine and Forensic Medicine, Henan University of Science and Technology, Luoyang, Henan, 471023, People's Republic of China, Tel +86 13698882699, Email lyxiangjun@126.com

Purpose: Dexmedetomidine (DEX) and esketamine (ESK) are often used together during anesthesia. This study aimed to establish a sensitive and reliable HPLC-MS/MS method for simultaneous quantification of ESK and its active metabolite norketamine (NORK) in beagle dog plasma and to investigate the pharmacokinetic drug-drug interactions (DDIs) between DEX and ESK/NORK.

Methods: A simple protein precipitation method using acetonitrile was applied for plasma sample preparation. After chromatographic separation, analytes were detected by HPLC-MS/MS in positive ion mode using multiple reaction monitoring (MRM). The mass transitions were m/z 238.10→125.10 for ESK, m/z 224.10→125.10 for NORK, and m/z 354.20→209.00 for the internal standard (proadifen). Six beagle dogs were intramuscularly administered 1 mg/kg ESK alone in the first period (ESK group). After a washout, the same dogs received intravenous DEX (2 μg/kg) for 7 consecutive days, followed by co-administration of ESK (DEX+ESK group). The pharmacokinetic parameters of ESK and NORK were calculated using DAS software. Independent-sample *t* test was used to compare the differences of pharmacokinetic parameters between ESK group and DEX+ESK group, and $P < 0.05$ indicated a statistically significant difference.

Results: Both ESK and NORK exhibited good linearity within the concentration range of 1–400 ng/mL, and the methodological validation met the requirements. When ESK was used in combination with DEX, the main pharmacokinetic parameters of ESK and NORK changed, the C_{max}, $AUC_{(0–24)}$ and $AUC_{(0-\infty)}$ of ESK increased, the C_{max}, of NORK decreased, and the $AUC_{(0–12)}$ and $AUC_{(0-\infty)}$ of NORK increased too.

Conclusion: A novel HPLC-MS/MS method was developed and validated and successfully applied to simultaneously quantify ESK and NORK in beagle dog plasma. The pharmacokinetic DDI results indicate that DEX could inhibit the metabolism of ESK, alter pharmacokinetic characteristics of ESK and its metabolite NORK, and significantly increase the systemic exposure of both ESK and NORK.

Keywords: dexmedetomidine, esketamine, norketamine, pharmacokinetics, drug-drug interactions, beagle dog

Introduction

Polypharmacy is a promising approach for treating diseases with complex symptoms. However, polypharmacy may lead to unforeseen drug-drug interactions (DDIs), potentially compromising therapeutic efficacy and triggering adverse drug reactions (ADRs).[1] DDIs represent a significant concern for clinical care and public health, but the health consequences of many DDIs remain largely underexplored. This knowledge gap underscores the critical need for pharmacoepidemiologic research to evaluate real-world health outcomes of DDIs.[2]

Received: 12 July 2025
Accepted: 18 September 2025
Published: 22 September 2025

The highly selective α2-adrenoceptor agonist dexmedetomidine (DEX) is a commonly used sedative drug for patients undergoing anesthesia and intensive care treatment.[3] DEX is increasingly being used in surgical patients requiring general anesthesia. Administration of DEX during surgery may significantly improve postoperative recovery quality and chronic pain.[4] DEX has a potent neuroprotective effect, including protecting the blood-brain barrier, reducing neuronal death, maintaining hemodynamic stability, and reducing postoperative agitation and cognitive dysfunction.[5] Meanwhile, DEX possesses anti-inflammatory properties that can counteract the pro-inflammatory mechanisms triggered by surgical injury, and simulate natural sleep pathways, thereby reducing the need for opioid medication and promoting the preservation of cognitive function.[6] Additionally, dexmedetomidine may play a promising and beneficial role in the treatment of cardiovascular disease.[7] DEX is a widely used sedative in clinic, after intravenous administration of DEX, it quickly distributes and is mainly metabolized by cytochrome P450 2A6 (CYP2A6).[8]

Esketamine (ESK, Figure 1a) is (S)-(+)-ketamine or (S)-ketamine, which was approved by the Food and Drug Administration and European Medicines Agency in 2019 for patients with treatment-resistant depression, and ESK is the only glutamatergic neuromodulator approved for enhancing the efficacy of selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs).[9,10] Globally, the use of ESK is increasing, as it is speculated that its S-(+)-enantiomer has an anesthetic and analgesic effect that is four times stronger than that of the R(-)-enantiomer, and approximately twice as strong as the racemic mixture of ketamine.[11] ESK have anesthetic and analgesic properties. During the anesthesia period of liposuction surgery, the use of subanesthetic doses of ESK (0.15–0.3 mg/kg/h) as an adjuvant drug can enhance the sedative and analgesic effects of DEX and remifentanil.[12] Intravenous administration of a single dose of ESK (0.25 mg/kg) before anesthesia induction can reduce the incidence of delirium in relatively young patients (ASA class II or III) undergoing cardiac surgery with cardiopulmonary bypass.[13] Among children undergoing tonsillectomy and/or adenoidectomy under sevoflurane anesthesia, intranasal DEX-ESK pretreatment can more effectively reduce emergence delirium compared to ESK alone.[14] Among elderly patients aged 65 and above undergoing hip fracture surgery, the adjunctive use of ESK in patient-controlled intravenous analgesia can improve depressive symptoms and elevate the levels of brain-derived neurotrophic factor and serotonin in the blood.[15] ESK undergoes oxidative metabolism, mainly to norketamine (NORK, Figure 1b) by cytochrome P450 (CYP) 3A4, CYP2B6 and CYP2C9 enzymes.[11,16]

DEX and ESK are commonly used anesthetic adjuvants and can be used simultaneously in clinical practice.[12,14] The use of intravenous ESK as an adjuvant in general anesthesia may represent a potentially beneficial strategy for reducing susceptibility to postnatal depression, with potential benefits for preventing postoperative depression and postoperative cognitive dysfunction.[17] Compared to the opioid-based anesthesia with propofol-remifentanil, the opioid-free anesthesia with ESK-DEX proved to be feasible for shoulder arthroscopy, resulting in a reduced incidence of postoperative nausea and vomiting postoperative nausea and vomiting and a shorter duration of stay in the post-anesthesia care unit.[18]

DEX is often co administered with ESK in animals and humans, so drug interactions are likely to occur and must be characterized.[19] Because both DEX and ESK are metabolized by CYP450 enzymes and DEX is an inhibitor of CYP3A4,[19] a pharmacokinetic DDI is likely when they are co-administered. In the current study, a sensitive HPLC-

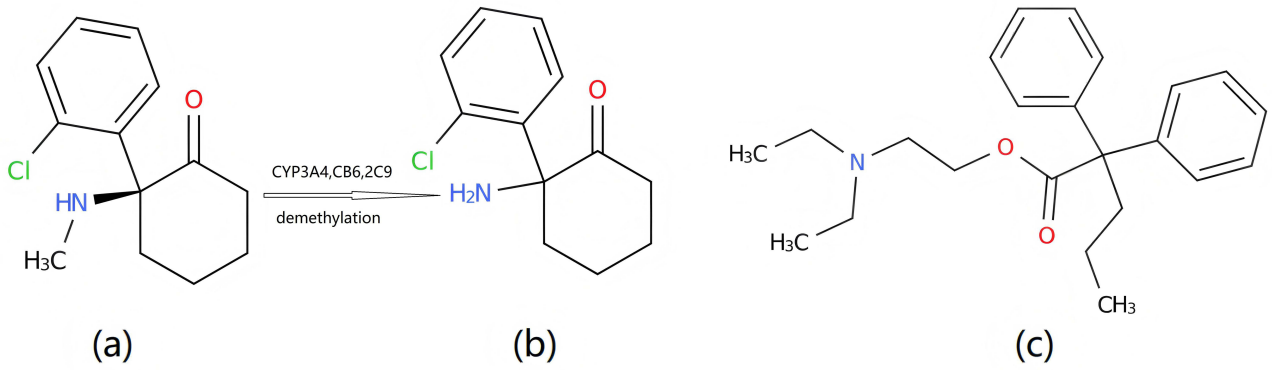

Figure 1 The chemical structure diagram of ESK (**a**), NORK (**b**) and IS (**c**).

MS/MS method was first developed for the simultaneous determination of ESK and NORK in beagle plasma, using proadifen as an internal standard (IS, Figure 1c). Then, the impact of DEX on the pharmacokinetics of ESK and NORK in beagle dogs was investigated.

Materials and Methods

Chemicals and Reagents

ESK (purity > 98%), NORK (purity > 98%) and proadifen (SFK-525A, purity > 98%, IS) were provided by Henan Yicheng Judicial Appraisal Center (Zhengzhou China). Methanol and acetonitrile produced by Kewell Chem LLC were HPLC grade and also met American Chemical Society Specifications (ACS specifications). Formic acid was procured from Sigma-Aldrich. ESK hydrochloride injection was purchased from Yangtze River Pharmaceutical Group Co., Ltd. DEX hydrochloride injection was purchased from Hunan Kelun Pharmaceutical Co., Ltd.

Instrumentation and Conditions

The HPLC instrument was Shimadzu LC-20 series, including LC-20AD pump, DGU-20A3r online deaerator, SIL-20A automatic sampler, CBM-20A signal receiver and Shimadzu chemistry workstation. The mass spectrometry was Shimadzu LCMS-8045 triple quadrupole mass spectrometer.

ESK, NORK, and IS were separated on a Shimadzu VP-ODS C18 chromatographic column (4.6 × 150 mm, 5 μm) with a mobile phase consisting of 0.1% formic acid (40%) and methanol (60%), at a column temperature of 30°C and a flow rate of 0.3 mL/min. Under positive ion conditions of electrospray ionization (ESI) source, the Multiple reaction monitoring (MRM) conditions of ESK, NORK and IS were shown in Table 1. The flow rate of atomizing gas was 3.0 L/min, the heating gas flow rate and drying gas flow rate were both 10.0 L/min. Labsolutions LCMS Workstation Software completed data collection and control.

Solutions Preparation

By accurately weighing 10 mg of ESK into a 10 mL volumetric flask, dissolving it with methanol, and diluting to volume, 1 mg/mL ESK stock solution was obtained. Using the same method, the stock solutions for NORK and IS were prepared. Then 100 μg/mL, 10 μg/mL and 1 μg/mL standard application solution of ESK and NORK were obtained through gradient dilution of the stock solution sequentially. Calibration curve solutions and quality control (QC) solutions were obtained by adding working solutions of different concentrations and volumes to different plasma samples. The final concentrations of ESK and NORK calibration curves were 1, 5, 10, 20, 50, 100, 200, and 400 ng/mL. The low QC, medium QC, and high QC concentrations of ESK and NORK were 2, 80, and 320 ng/mL, respectively. A certain amount of 1 mg/mL proadifen IS solution was diluted with acetonitrile to prepare a 20 ng/mL IS working solution. The stock solutions and application solutions of ESK, NORK, and IS were stored in a refrigerator at 4°C. The plasma standard solutions (standard curve samples and QC samples) were frozen and stored at −20°C.

Sample Preparation

The method of acetonitrile precipitation of plasma protein was been applied to the extraction of plasma drug. The frozen samples to be tested were thawed at room temperature before detection. Accurately pipette 100 μL of plasma into a 1.5 mL EP tube, add 250 μL of internal standard working solution (concentration of 20 ng/mL, acetonitrile solution)

Table 1 MS Parameters of ESK, NORK and IS

Analytes	Formula Weight	ESI Source	Quantitative Ion (m/z)	Qualitative Ion (m/z)
ESK	237.73	+	238.10>125.10	238.10>220.10
NORK	223.66	+	224.10>125.10	224.10>179.10
IS	179.26	+	354.20>209.00	354.20>105.00

Note: + indicates that this study ionizes and detects drug samples in positive ion mode.

https://doi.org/10.2147/DDDT.S553389

and vortex for 15 seconds. Centrifuge the mixture at 10,000 r/min for 15 minutes, transfer the supernatant to a sample vial, and inject 2 μL of the supernatant.

Method Validation

According with the guidelines for validation of quantitative analysis methods for biological samples in the Chinese Pharmacopoeia (2020 Edition),[20] the HPLC-MS/MS method was validated including the specificity, calibration curve, lower limit of quantitation (LLOQ), precision and accuracy, recovery, matrix effect (ME) and stability.

Selectivity

Blank plasma from 6 beagle dogs was used to demonstrate selectivity. The response of acceptable interference components should be below 20% of the LLOQ response of ESK and NORK, and below 5% of the IS response.

Calibration Curves and LLOQ

A series of concentrations (1, 5, 10, 20, 50, 100, 200 and 400 ng/mL) of ESK and NORK plasma standard samples were prepared and processed according to the plasma sample processing method, then detected. The peak areas of ESK or NORK and the peak area of the IS were recorded as A_s and A_i, respectively. The calibration curve was plotted with the ordinate (y, the ratio of A_s/A_i) and the abscissa (x, the concentration of ESK or NORK). The lowest concentration of the calibration curve of ESK or NORK was defined as the LLOQ.

Accuracy and Precision

The accuracy and precision were assessed by the determination of QC samples (2, 80 and 320 ng/mL) in six replicates. On the same day, the intra-day precision (RSD%) and accuracy (%) were calculated, and the inter-day precision (RSD%) and accuracy (%) were calculated by continuous measurement within 3 days.

Recovery and Matrix Effects

The QC samples (2, 80 and 320 ng/mL) were prepared. The recoveries were calculated by comparing the peak area of the conventional pretreated QC sample with the peak area after extraction of the corresponding concentration of blank plasma (after extraction). The matrix effects should be obtained by calculating the ratio of peak area in the presence of matrix to the corresponding peak area in the absence of matrix.

Stability

Stability tests were conducted under four different storage conditions at three quality control levels (2, 80, and 320 ng/mL): 12 hours at room temperature, 4 weeks at −20°C, and after three freeze-thaw cycles (−20 to 25°C), the processed samples left for 12 hours.

Animals

Six beagle dogs weighing 8–10 kg with animal production license number SCXK (Hubei) 2021–002 were purchased from Hubei Yizhicheng Biotechnology Co., Ltd. The experiment obtained the necessary approval from the Animal Laboratory of Henan University of Science and Technology (202407003). The experiment was approved according to the Laboratory animals-guidelines for ethical review of welfare (GB/T 35892–2018).

DDI Study

Before the test, the six beagle dogs had free access to water but were fasted for 12 h. Blood samples (0.5 mL) were collected into heparinized tubes before administration and 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 h after intramuscular injection of 1.0 mg/kg ESK (ESK group). The plasma was separated after centrifugation at 3000 rpm for 10 min and stored at −20°C until it was detected.

After one week, the same six beagle dogs were injected intravenously slowly with 2 μg/kg DEX, continuous injection for 7 days. On the seventh day, 0.5 hour after intravenous injection of DEX, the six beagle dogs were given by intramuscular injection with ESK 1.0 mg/kg. Blood samples (0.5 mL) were collected into heparinized tubes before

administration and 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 h after injection of ESK (DEX + ESK group). The plasma was also separated after centrifugation at 3000 rpm for 10 min and stored at −20°C until it was detected.

The above-developed HPLC-MS/MS method was used to simultaneously detect ESK and NORK in beagle dog plasma. Samples with drug concentrations exceeding the upper limit of the standard curve shall be diluted with blank matrix, reprocessed, and tested, and the results shall be multiplied by the dilution factor. The pharmacokinetic parameters of ESK and NORK were calculated using Drug And Statistics software (DAS version 2.0) with statistical moment, including C_{max},T_{amx}, $t_{1/2}$, Vd, Cl, AUC. The calculation process of pharmacokinetic parameters was as follows: Open the DAS software, select the batch data analysis of the pharmacokinetic module, choose non intravenous administration method, enter the plasma drug concentration at the corresponding time point, and click run.

Independent-sample *t* test was used to compare the differences of pharmacokinetic parameters between ESK group and DEX + ESK group, and $P < 0.05$ indicated a statistically significant difference.

Results

Results of MS

The molecular weights of ESK, NORK, and IS are 237.73, 223.66, and 353.50, respectively. Under ESI positive ion conditions, the parent particles obtained through protonation are 238.10, 224.15 and 354.20, respectively. The characteristic daughter ions of ESK are 221.10 and 125.10, the characteristic daughter ions of NORK are 179.10 and 125.10, and the characteristic daughter ions of IS are 209.00 and 105.00. The characteristic daughter ion fragments of ESK, NORK, and IS were shown in Figure 2. In ESI+ mode, the analytes were ionized by protonation and detected by MRM, and the MS qualitative diagrams were shown in Figure 3.

Figure 2 The characteristic daughter ion fragments of esketamine (**a**), norketamine (**b**), and IS (**c**).

https://doi.org/10.2147/DDDT.S553389

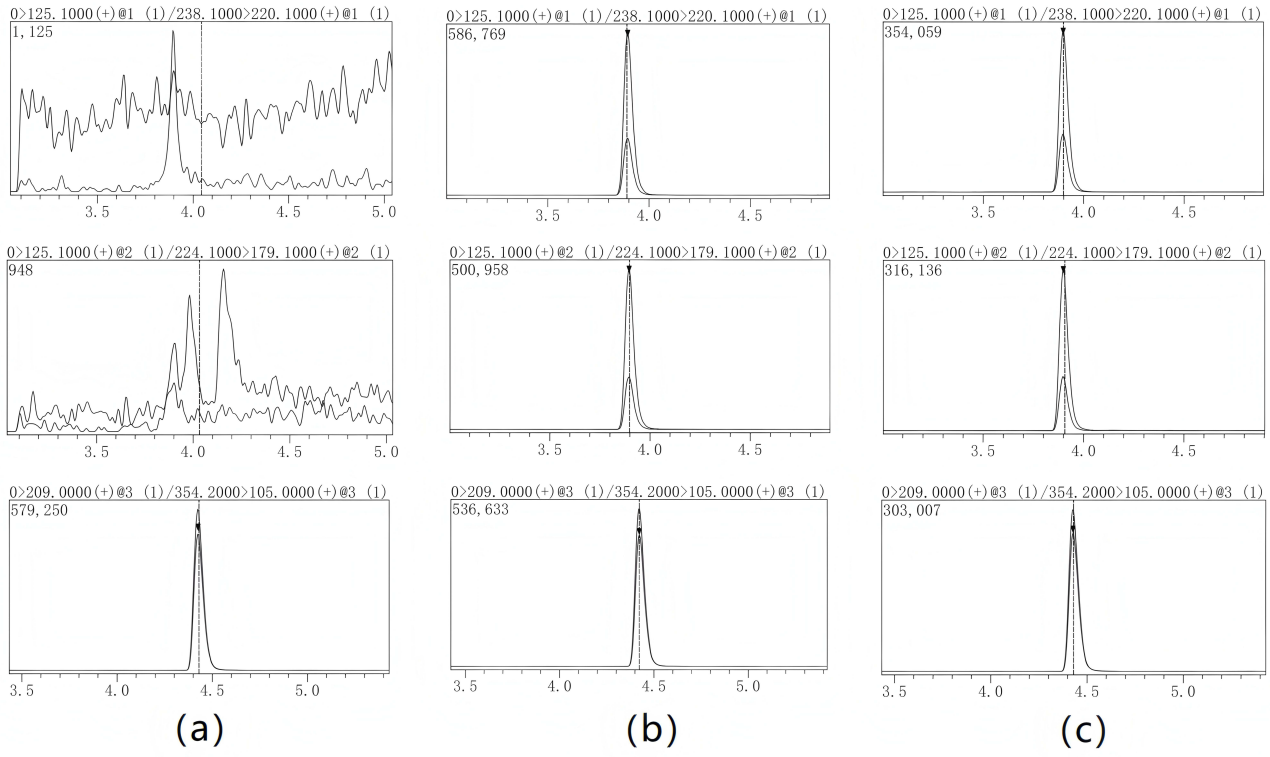

Figure 3 MS qualitative diagram of esketamine, norketamine and IS. (**a**)-blank plasma+IS, (**b**)-blank plasma + ESK, NORK and IS, (**c**)- beagle dog plasma sample.

Results of Method Validation

Specificity

ESK, NORK and IS were well separated from endogenous substances under the above experimental conditions. Representative chromatograms of a blank plasma sample spiked with IS (A), a blank plasma sample spiked with ESK, NORK and IS (B), and a beagle sample after administration (C) were shown in Figure 4. The retention times of ESK, NORK and IS were 3.896, 3.896 and 4.428 min, respectively.

Linearity

The regression equations, correlation coefficients (R^2), and linear ranges of ESK and NORK were shown in Table 2. Within the range of 1–400 ng/mL, both ESK and NORK exhibited good linearity, with LLOQ values of 1.00ng/mL for both ESK and NORK.

Precision and Accuracy

Table 3 showed the results of intra-day and inter-day precision and accuracy for ESK and NORK. In QC samples (2, 80, and 320 ng/mL), the precision (RSD%) of ESK and NORK did not exceed 8.13%, and the accuracy ranged from 96.83% to 103.25%.

Recovery and ME

The extraction recovery rates of QC samples of ESK (2, 80 and 320 ng/mL) were all higher than 80.81%, and the ME ranged from 101.50% to 103.80%. The extraction recovery rates of QC samples of NORK (2, 80 and 320 ng/mL) were all higher than 80.16%, and the ME ranged from 97.49% to 102.77%.

Stability

The stability of ESK and NORK in beagle plasma was evaluated under the four different conditions mentioned above. The stability test results were shown in Table 4. From the results, it could be seen that ESK and NORK were stable under four experimental conditions.

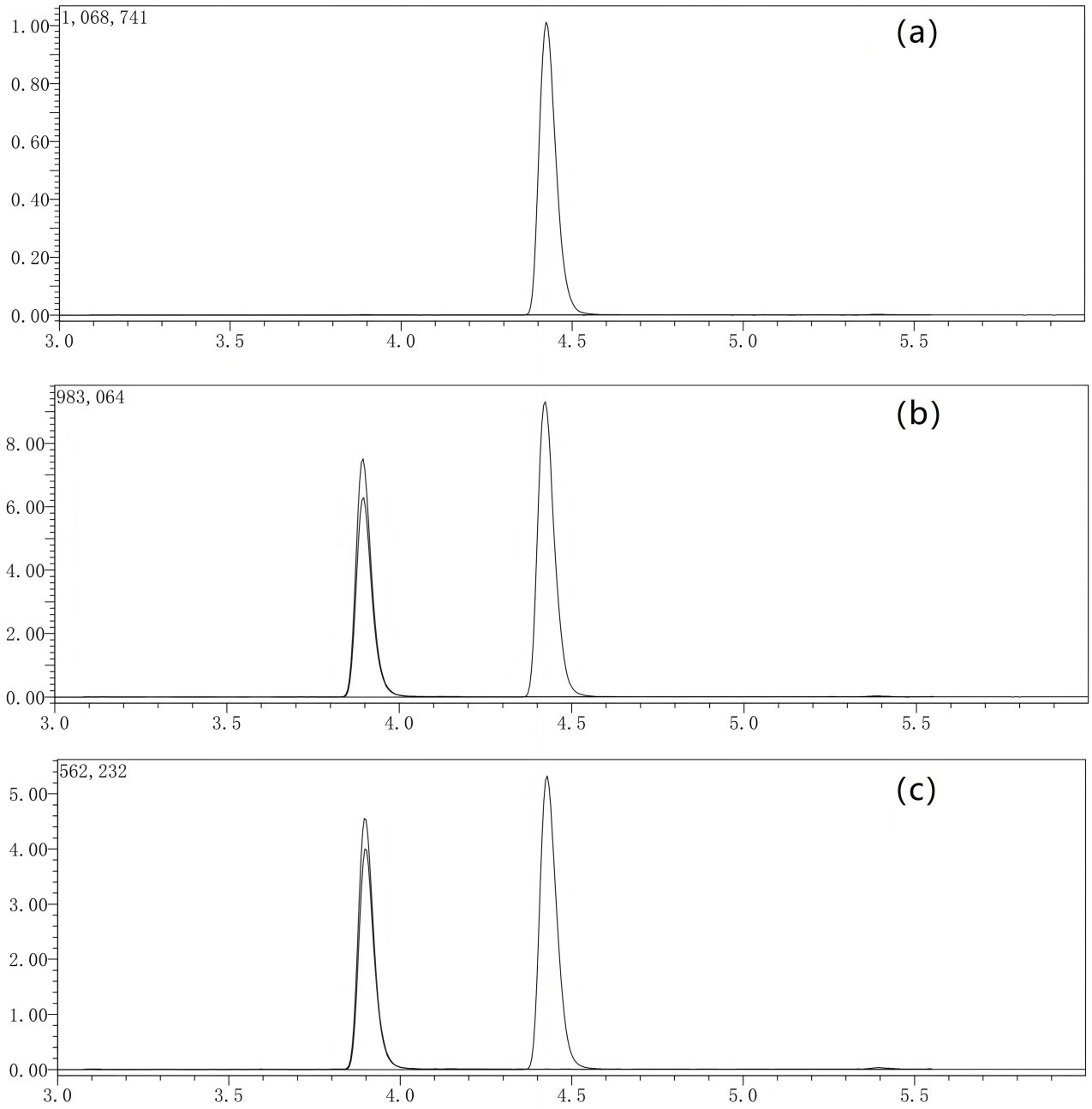

Figure 4 Typical MS chromatogram of ESK, NORK and IS. (**a**) Blank plasma + IS, (**b**) blank plasma + ESK (100 ng/mL), NORK (100 ng/mL) and IS, (**c**) a beagle dog sample after administration.

Results of the Effect of DEX on ESK and NORK

The ESK plasma concentration time curves of the ESK group and the DEX+ESK group were shown in Figure 5, and the NORK plasma concentration time curves of the ESK group and the DEX+ESK group were shown in Figure 6. The main

Table 2 The Regression Equation, Linear Ranges, R^2 and LLOQ of ESK and NORK

Analytes	Regression Equation	Linear Ranges (ng/mL)	R^2	LLOQ (ng/mL)
ESK	$y = 0.0281\ x - 0.0108$	1–400	0.999 7	1
NORK	$y = 0.0174\ x + 0.0419$	1–400	0.999 6	1

https://doi.org/10.2147/DDDT.S553389

Table 3 Precision and Accuracy of ESK and NORK in Beagle Dog Plasma (n = 6, Mean ± SD)

Analytes	Spiked (ng/mL)	Intra-Day		Inter-Day	
		RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
ESK	1	6.76	96.83	8.13	99.56
	2	5.94	102.67	6.19	101.47
	80	2.94	103.25	4.97	101.04
	320	2.13	98.88	2.54	99.71
NORK	1	8.21	98.67	8.28	97.72
	2	5.79	102.33	5.70	102.81
	80	4.65	97.49	4.96	101.47
	320	2.26	102.77	3.24	99.58

Table 4 The Stability of ESK and NORK in Beagle Plasma (n = 6, Mean ± SD)

Analytes	Spiked (ng/mL)	Room Temperature, 12 h		Autosampler 4 °C, 12 h		Three Freeze-Thaw		−20°C, 4 Weeks	
		RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
ESK	2	3.04	95.17	3.74	104.08	3.91	97.42	4.77	97.42
	80	2.48	103.38	3.44	96.35	3.37	103.59	3.49	96.02
	320	2.70	101.03	1.77	98.49	1.24	101.54	1.72	98.14
NORK	2	5.81	97.58	4.66	101.83	6.24	98.25	4.15	98.67
	80	4.28	98.84	3.84	97.79	3.36	102.68	3.41	101.45
	320	1.22	102.85	2.36	98.97	2.35	101.09	2.94	98.82

pharmacokinetic parameters of ESK and NORK were calculated using the DAS 2.0 program, as shown in Tables 5 and 6, respectively.

The results showed that the C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ of ESK in the DEX+ESK group were 53.66%, 117.38%, and 114.70% higher than those in the ESK group, respectively. The $t_{1/2}$ was slightly prolonged, Vz/F increased, and CLz/F decreased accordingly. Meanwhile, the C_{max} of NORK in the DEX+ESK group was 12.75% lower than that in the ESK

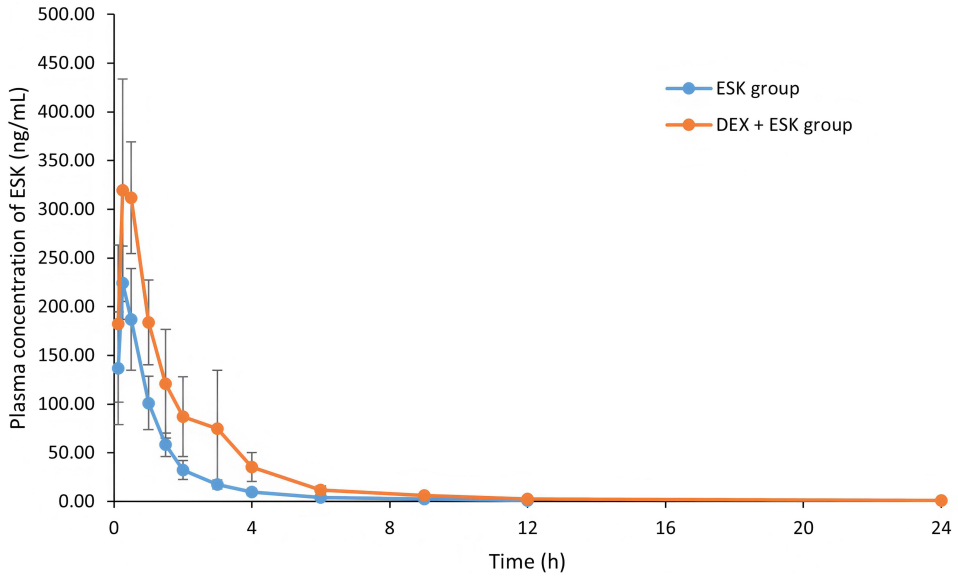

Figure 5 Mean drug concentration-time curves of ESK in ESK group and DEX+ESK group after intramuscular injection of 1.0 mg/kg ESK to six beagle dogs.

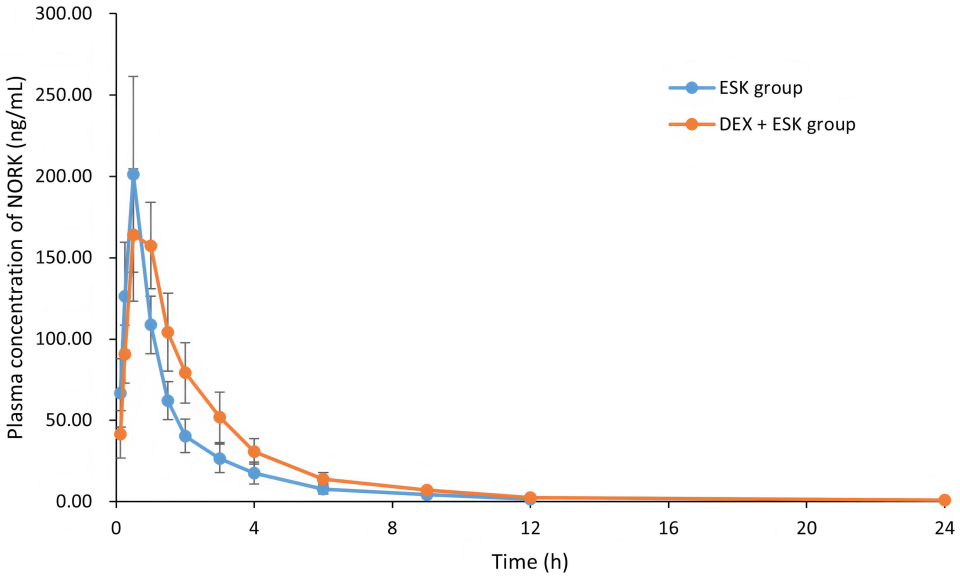

Figure 6 Mean drug concentration-time curves of NORK in ESK group and DEX+ESK group after intramuscular injection of 1.0 mg/kg ESK to six beagle dogs.

group, but the $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ of NORK in the DEX+ESK group were 45.27% and 43.17% higher than those in the ESK group, respectively. The $t_{1/2}$ was prolonged from 2.38 hours to 3.44 hours, and Vz/F and CLz/F were correspondingly reduced. DEX might affect the pharmacokinetics of ESK and NORK in beagle dogs, and inhibit the metabolism of ESK in beagle dogs and increase the exposure of ESK and NORK.

Table 5 Pharmacokinetic Parameters of ESK in ESK Group and DEX+ESK Group After Intramuscular Injection of 1.0 mg/kg ESK (n = 6, Mean ± SD)

Parameters	ESK Group	DEX+ESK Group
C_{max} (ng/mL)	249.22 ± 28.25	382.96 ± 63.02**
T_{max} (h)	0.27 ± 0.12	0.38 ± 0.14
$t_{1/2}$ (h)	2.84 ± 0.93	3.05 ± 1.27
CLz/F (L/h/kg)	3.54 ± 0.54	1.69 ± 0.39**
Vz/F (L/kg)	14.62 ± 6.09	7.16 ± 2.46*
$AUC_{(0\text{–}24)}$ (ng·h/mL)	284.50 ± 50.04	618.45 ± 145.46**
$AUC_{(0\text{-}\infty)}$ (ng·h/mL)	288.99 ± 51.78	620.47 ± 148.59**

Notes: *indicate a significant difference compared with the blank group, $P < 0.05$; **indicate a significant difference compared with the model group, $P < 0.01$.

Table 6 Pharmacokinetic Parameters of NORK in ESK Group and DEX+ESK Group After Intramuscular Injection of 1.0 mg/kg ESK (n = 6, Mean ± SD)

Parameters	ESK Group	DEX+ESK Group
C_{max} (ng/mL)	207.94 ± 53.66	181.43 ± 27.56
T_{max} (h)	0.46 ± 0.10	0.75 ± 0.27
$t_{1/2}$ (h)	2.50 ± 0.63	2.96 ± 1.28
CLz/F (L/h/kg)	3.20 ± 0.43	2.28 ± 0.52*
Vz/F (L/kg)	11.58 ± 3.39	9.60 ± 3.82
$AUC_{(0\text{–}24t)}$ (ng·h/mL)	311.07 ± 42.60	451.89 ± 80.67*
$AUC_{(0\text{-}\infty)}$ (ng·h/mL)	317.24 ± 43.60	454.19 ± 81.93*

Note: *indicate a significant difference compared with the ESK group, $P < 0.05$.

https://doi.org/10.2147/DDDT.S553389

Discussion

Method Development

Before establishing the methodology, the physicochemical properties of ESK and NORK were first determined, including polarity, stability pKa, molecular weight, fragment ions, etc. Based on the polarity of ESK and NORK, a reverse phase chromatography column was selected to separate the test substance, Shimadzu VP-ODS C18 was considered a suitable chromatographic column for testing the retention time, peak shape, and resolution of different fixed relative target substances, as well as evaluating column efficiency (theoretical plate number) and column pressure. The mobile phase adopted a system of methanol (60%) and 0.1% formic acid aqueous solution (40%),[21] and formic acid was added to improve peak shape and ionization efficiency. Under the condition of isocratic mobile phase, the chromatographic peaks were good. The choice of flow rate directly affects the sensitivity and accuracy of detection. Excessive flow rate can lead to a decrease in ionization efficiency and affect detection sensitivity; if the flow rate is too slow, it may lead to enhanced matrix effects and affect the accuracy of detection. Low flow rate not only improves separation efficiency but also enhances the sensitivity and accuracy of mass spectrometry detection. Therefore, the flow rate for this study was 0.3 mL/min.

Proadifen (SFK-525A) was a recommended IS for determination of poisons (drugs) in blood and urine by liquid chromatography mass spectrometry (SF/T 0175–2024).[22] Under the experimental conditions, the peak shape of IS was good, with peak appearing after ESK and NORK without interfering with each other. Therefore, proadifen (SFK-525A) was used as IS.

The method of precipitation of plasma proteins is a commonly used pre-treatment method for plasma samples, which has the advantages of being simple, fast, and environmentally friendly.[23,24] This simplified the sample pre-treatment procedure to save time and cost without affecting the LLOQ.[23] Therefore, in this study the plasma sample was pretreated using acetonitrile precipitation of plasma proteins, and matrix interference was removed to improve ionization efficiency.

DDIs

The use of combination drugs among patients is increasing due to effectiveness compared to monotherapies.[25] DDIs occur when multiple medications are co-administered, and DDIs are associated with increased or decreased adverse effects and increased or decreased therapeutic effects.[26,27] When drugs alter the absorption, transport, distribution, metabolism, or excretion of co administered drugs, pharmacokinetic DDIs occur. The occurrence of pharmacokinetic DDIs may lead to an increase or decrease in drug concentration, which significantly affects the efficacy and safety of the drug in patients.[28]

DEX is a highly selective α_2 adrenergic receptor agonist with sedative, analgesic, anxiolytic, and sympatholytic properties, DEX may play a promising and beneficial role in the treatment of cardiovascular disease.[7] However, DDIs related to dexmedetomidine also occur. In terms of pharmacodynamics, drugs used in combination with dexmedetomidine have more frequent occurrences of bradycardia than expected, including Lactated Ringer's solution, bupivacaine, and risperidone.[29] In children who underwent tonsillectomy and/or adenoidectomy and were anesthetized with sevoflurane, intranasal DEX-ESK pre administration was more effective in reducing delirium, improving sedative effects, shortening the onset time, and increasing parental satisfaction without significant adverse reactions.[14] In terms of pharmacokinetics, DEX can inhibit the metabolism of valdecoxib in beagles and increase the exposure of valdecoxib, but it does not affect the pharmacokinetics of parecoxib.[30] DEX could inhibit the metabolism of dezocine and midazolam, increase the exposure of dezocine and midazolam in beagle dogs.[31] DEX can inhibit CYP 3A4 and may produce sufficient liver concentration to interfere with the metabolism of tacrolimus.[32]

In this study, after the combination of ESK and DEX, the C_{max} and AUC of ESK significantly increased, while VZ and CL decreased, indicating a slowdown in ESK metabolism and an increase in ESK exposure. The active metabolite NORK of ESK showed a slight decrease in C_{max}, while AUC significantly increased, VZ and CL both decreased, indicating a slowdown in NORK metabolism and an increase in NORK exposure.

CYP enzymes dominate the metabolism of numerous endogenous and xenobiotic substances. ESK is metabolized in the liver through CYP2B6, CYP3A4, and CYP2C9 isoforms as its main active metabolite NORK,[16] and NORK is further metabolized to five diastereomeric hydroxynorketamines by hydroxylation of the cyclohexyl ring.[33] Due to the inhibitor

of CYP3A4 by DEX, which is also a strong inhibitor of canine liver microsomes,[19,32] DEX not only affects the metabolism of ESK but also affects the metabolism of NORK, resulting in an increase in plasma exposure of ESK and an increase in exposure of NORK. Therefore, the pharmacological effects of ESK and NORK are also enhanced.

Due to its effectiveness compared to monotherapy, the use of combination drugs in patients is increasing. However, healthcare providers should continue to pay attention to potential risks related to patient safety caused by DDIs when using combination drugs.[25]

Conclusion

A novel HPLC-MS/MS method was developed and validated and successfully applied to simultaneously quantify ESK and NORK in beagle dog plasma. The pharmacokinetic DDI results indicate that DEX could inhibit the metabolism of ESK, alter pharmacokinetic characteristics of ESK and its metabolite NORK, and significantly increase the systemic exposure of both ESK and NORK.

Data Sharing Statement

All data generated in the present study may be requested from the corresponding author Xiangjun Qiu by Email lyxiangjun@126.com.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Henan Medical Science and Technology Research and Development Program Project in 2024 (LHGJ20240782).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Jung S, Yoo S. Interpretable prediction of drug-drug interactions via text embedding in biomedical literature. *Comput Biol Med*. 2025;185:109496. doi:10.1016/j.compbiomed.2024.109496
2. Chen C, Pham Nguyen TP, Hughes JE, et al. Evaluation of drug-drug interactions in pharmacoepidemiologic research. *Pharmacoepidemiol Drug Saf*. 2025;34(1):e70088. doi:10.1002/pds
3. Duan S, Zhou S. Dexmedetomidine and perioperative arrhythmias. *J Cardiothorac Vasc Anesth*. 2024;38(5):1221–1227. doi:10.1053/j.jvca.2024.01.006
4. Verret M, Le JBP, Lalu MM, et al. Effectiveness of dexmedetomidine on patient-centred outcomes in surgical patients: a systematic review and Bayesian meta-analysis. *Br J Anaesth*. 2024;133(3):615–627. doi:10.1016/j.bja.2024.06.007
5. Tao Z, Li P, Zhao X. Progress on the mechanisms and neuroprotective benefits of dexmedetomidine in brain diseases. *Brain Behav*. 2024;14(11):e70116. doi:10.1002/brb3.70116
6. Strada AL, Tevay A, Scoggins M, Gonzalez K. Dexmedetomidine for postoperative delirium prevention in the older adult: an integrative review. *AANA J*. 2024;92(5):383–389.
7. Jiang L, Xiong W, Yang Y, Qian J. Insight into cardioprotective effects and mechanisms of dexmedetomidine. *Cardiovasc Drugs Ther*. 2024;38(6):1139–1159. doi:10.1007/s10557-024-07579-9
8. Wang L, Wang S, Qi J, et al. Impact of CYP2A6 gene polymorphism on the pharmacokinetics of dexmedetomidine for premedication. *Expert Rev Clin Pharmacol*. 2018;11(9):917–922. doi:10.1080/17512433.2018.1510312
9. Kawczak P, Feszak I, Bączek T. Ketamine, esketamine, and arketamine: their mechanisms of action and applications in the treatment of depression and alleviation of depressive symptoms. *Biomedicines*. 2024;12(10):2283. doi:10.3390/biomedicines12102283
10. Vekhova KA, Namiot ED, Jonsson J, Schiöth HB. Ketamine and esketamine in clinical trials: FDA-approved and emerging indications, trial trends with putative mechanistic explanations. *Clin Pharmacol Ther*. 2025;117(2):374–386. doi:10.1002/cpt.3478
11. Peltoniemi MA, Hagelberg NM, Olkkola KT, Saari TI. Ketamine: a review of clinical pharmacokinetics and pharmacodynamics in anesthesia and pain therapy. *Clin Pharmacokinet*. 2016;55(9):1059–1077. doi:10.1007/s40262-016-0383-6

12. Chen H, Zhi J, Wang L, et al. Subanesthetic dose of esketamine improves the sedative and analgesic effects of dexmedetomidine and remifentanil in liposuction anesthesia: a prospective, double-blinded, randomized controlled trial. *Drug Des Devel Ther*. 2024;18:3645–3658. doi:10.2147/DDDT.S470891
13. Xiong X, Shao Y, Chen D, Chen B, Lan X, Shi J. Effect of esketamine on postoperative delirium in patients undergoing cardiac valve replacement with cardiopulmonary bypass: a randomized controlled trial. *Anesth Analg*. 2024;139(4):743–753. doi:10.1213/ANE.0000000000006925
14. Liao Y, Xie S, Zhuo Y, et al. Intranasal dexmedetomidine-esketamine combination premedication versus monotherapy for reducing emergence delirium and postoperative behavioral changes in pediatric tonsillectomy and/or adenoidectomy: a randomized controlled trial. *Drug Des Devel Ther*. 2024;18:4693–4703. doi:10.2147/DDDT.S488706
15. Cai J, Chen X, Jin Z, Chi Z, Xiong J. Effects of adjunctive esketamine on depression in elderly patients undergoing hip fracture surgery: a randomized controlled trial. *BMC Anesthesiol*. 2024;24(1):340. doi:10.1186/s12871-024-02733-0
16. Schep LJ, Slaughter RJ, Watts M, Mackenzie E, Gee P. The clinical toxicology of ketamine. *Clin Toxicol (Phila)*. 2023;61(6):415–428. doi:10.1080/15563650.2023.2212125
17. Lin X, Liu X, Huang H, Xu X, Zhang T, Gao J. Esketamine and neurocognitive disorders in adult surgical patients: a meta-analysis. *BMC Anesthesiol*. 2024;24(1):448. doi:10.1186/s12871-024-02803-3
18. Xue Z, Yan C, Liu Y, et al. Opioid-free anesthesia with esketamine-dexmedetomidine versus opioid-based anesthesia with propofol-remifentanil in shoulder arthroscopy: a randomized controlled trial. *BMC Surg*. 2024;24(1):228. doi:10.1186/s12893-024-02518-9
19. Sandbaumhüter FA, Theurillat R, Thormann W. Effects of medetomidine and its active enantiomer dexmedetomidine on N-demethylation of ketamine in canines determined in vitro using enantioselective capillary electrophoresis. *Electrophoresis*. 2015;36(21–22):2703–2712. doi:10.1002/elps.201500147
20. The guidelines for validation of quantitative analysis methods for biological samples. National Pharmacopoeia Commission. *Chinese Pharmacopoeia (2020 edition)*. 2020; Part II: 466–472.
21. M Z Sharkawi M, H Amin N, R Mohamed N, S Zaki S, R Hemeda L. Novel environmental and sustainable approach for concurrent assay of antineoplastics in VMP regimen with a comprehensive pharmacokinetic study. *Sci Rep*. 2025;15(1):29894. doi:10.1038/s41598-025-15078-6
22. Determination of 238 poisons (drugs) in blood and urine by liquid chromatography mass spectrometry. *Judicial Administration Industry Standard of the People's Republic of China (SF/T 0175-2024)*. Ministry of Justice of the People's Republic of China; 2024.
23. Zhang N, Xu Y, Liang B, Zeng J, Wang R, Cai Y. A rapid and simple HPLC-MS/MS method for the quantitative determination of colistin for therapeutic drug monitoring in clinical practice. *Drug Des Devel Ther*. 2024;18:4877–4887. doi:10.2147/DDDT.S479329
24. Bi G, Yu Q, Guo J, et al. Development and validation of a HPLC-MS/MS method for the determination of compound 13b in rat plasma and its application to a pharmacokinetic study. *Biomed Chromatogr*. 2025;39(4):e70044. doi:10.1002/bmc.70044
25. Jeong YU, Choi J, Park N, Ryu JY, Kim YR. Predicting drug-drug interactions: a deep learning approach with GCN-based collaborative filtering. *Artif Intell Med*. 2025;167:103185. doi:10.1016/j.artmed.2025.103185
26. Lu P, Zheng L, Lin J, et al. MSMDL-DDI: multi-layer soft mask dual-view learning for drug-drug interactions. *Comput Biol Chem*. 2025;115:108355. doi:10.1016/j.compbiolchem.2025.108355
27. Hailesilase GG, Kidane AM, Gebrezgabiher HA. Potential drug-drug interactions and associated factors among hospitalized pediatric patients in Adigrat general hospital, Tigrai, north Ethiopia: a retrospective cross-sectional study. *BMC Pediatr*. 2024;24(1):652. doi:10.1186/s12887-024-05128-9
28. Peng Y, Cheng Z, Xie F. Evaluation of pharmacokinetic drug-drug interactions: a review of the mechanisms, in vitro and in silico approaches. *Metabolites*. 2021;11(2):75. doi:10.3390/metabo11020075
29. Morris R, Kuppa SA, Zhu X, Bu K, Han W, Cheng F. The association between dexmedetomidine and bradycardia: an analysis of FDA Adverse Event Reporting System (FAERS) data and transcriptomic profiles. *Genes*. 2025;16(6):615. doi:10.3390/genes16060615
30. Hu J, Lv BF, Guo WJ, et al. Effects of dexmedetomidine on the pharmacokinetics of parecoxib and its metabolite valdecoxib in beagles by UPLC-MS/MS. *Biomed Res Int*. 2020;2020:1563874. doi:10.1155/2020/1563874
31. Zhou W, Li SL, Zhao T, et al. Effects of dexmedetomidine on the pharmacokinetics of dezocine, midazolam and its metabolite 1-hydroxymidazolam in beagles by UPLC-MS/MS. *Drug Des Devel Ther*. 2020;14:2595–2605. doi:10.2147/DDDT.S254055
32. Stiehl SR, Squires JE, Bucuvalas JC, Hemmelgarn TS. Tacrolimus interaction with dexmedetomidine--a case report. *Pediatr Transplant*. 2016;20(1):155–157. doi:10.1111/petr.12618
33. Toki H, Yamaguchi JI, Mizuno-Yasuhira A, Endo H. Chiral LC-MS/MS method for the simultaneous determination of (R,S)-ketamine, (R,S)-norketamine, and (2R,6R;2S,6S)-hydroxynorketamine in mouse plasma and brain. *J Pharm Biomed Anal*. 2023;224:115168. doi:10.1016/j.jpba.2022.115168